Technical Data Sheet

APC Magnetic Particles - DM

Product Information

Material Number: **557932**

Size: 5.0 ml

Clone: E30-221

Storage Buffer: Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

BD IMag™ Anti-Allophycocyanin (APC) Particles - DM are magnetic nanoparticles that have monoclonal antibody conjugated to their surfaces. These particles are optimized for the positive selection or depletion of leukocyte subpopulations using the BD IMagnet™ (Cat. No. 552311). The E30-221 antibody reacts with APC, a commonly used fluorochrome for flow cytometry. The binding of the E30-221 antibody to APC does not quench the fluorescence of the APC molecule.

Preparation and Storage

Store undiluted at 4° C.

Catalog Number	Antibody Clone	Antibody Specificity	BD IMag Streptavidin Particle Concentration	Tissue Used
555342	HIT3a	Human CD3	50 µl/10^7 total cells	PBMC
555415	HIB19	Human CD19	50 µl/10^7 total cells	PBMC
553051	RM4-5	Mouse CD4 (L3T4)	20 µl/10^7 total cells	Spleen
553035	53-6.7	Mouse CD8a (Ly-2)	10 µl/10^7 total cells	Spleen
553312	M1/70	Mouse CD11b (Integrin α_M chain)	50 µl/10^7 total cells	Bone Marrow
553092	RA3-6B2	Mouse CD45R/B220	20 µl/10^7 total cells	Spleen
553007	53-2.1	Mouse CD90.2 (Thy-1.2)	20 µl/10^7 total cells	Spleen
553129	RB6-8C5	Ly-6G and Ly-6C (Gr-1)	20 µl/10^7 total cells	Bone Marrow

Optimal concentrations of BD IMag™ Anti-APC Particles - DM for positive selection with some APC-conjugated monoclonal antibodies to human and mouse leukocyte antigens.

Application Notes

Application

Cell separation	Routinely Tested

Recommended Assay Procedure:

A detailed Magnetic Labeling and Separation Protocol follows. In brief, cells are labeled with the APC-conjugated antibody, which recognizes the subpopulation of interest. After washing away excess antibody, BD IMag™ Anti-APC Particles - DM are added to the cell suspension and bind the APC-conjugated antibody on the cells. The tube containing this labeled cell suspension is then placed within the magnetic field of the BD IMagnet™. Positive selection or depletion is then performed. Labeled cells migrate toward the magnet (positive fraction), leaving the unlabeled cells in suspension so they can be drawn off (depleted or negative fraction). The tube is then removed from the magnetic field for resuspension of the positive fraction. The selections are repeated twice to increase the purity of the positive fraction and the yield of the depleted fraction. The magnetic separation steps are diagrammed in the accompanying Depletion and Positive Selection Flow Charts. The small size of the BD IMag™ particles allows the positive fraction to be further evaluated in downstream applications such as flow cytometry.

MAGNETIC LABELING AND SEPARATION PROTOCOL

1. Prepare buffers and place on ice.
   a. Cell-staining buffer: Phosphate Buffered Saline, 3% heat inactivated fetal calf serum, 0.1% sodium azide.
   b. 1X BD IMag™ buffer: Dilute BD IMag™ Buffer (10X) (Cat. No. 552362) 1:10 with sterile distilled water or prepare Phosphate Buffered Saline, supplemented with 0.5% BSA, 2 mM EDTA, and 0.1% sodium azide.*
2. Aseptically prepare a single-cell suspension from the lymphoid tissue of interest or prepare PBMC from anti-coagulated blood, preferably by density gradient centrifugation using the appropriate density Ficoll-Paque solution. Remove clumps of cells and/or debris by passing the suspended cells through a 70-µm nylon cell strainer.
3. Count the cells, and resuspend them in cell-staining buffer at a concentration of 2 x 10e7 cells/ml.

BD Biosciences

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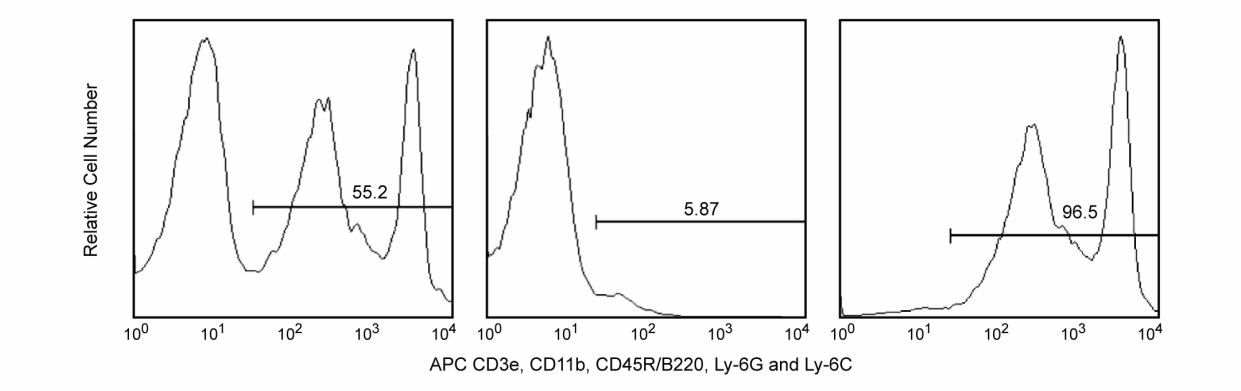
4. Optional: If needed, add BD Mouse Fc Block™ purified anti-mouse CD16/CD32 mAb 2.4G2 (Cat. No. 553141/553142) or BD Rat Fc Block™ purified anti-rat CD32 mAb D34-485 (Cat. No. 550270/550271) at 0.25 µg/10e6 cells, and incubate on ice for 15 minutes.
5. Add the APC-conjugated antibody (or cocktail of APC-conjugated antibodies) at the appropriate concentration, and incubate on ice for 15 minutes. **
6. Wash the labeled cells with an excess volume of 1X BD IMag™ buffer, and carefully aspirate ALL the supernatant. For depletions, proceed with Step 7. For positive selections, proceed with Step 18.

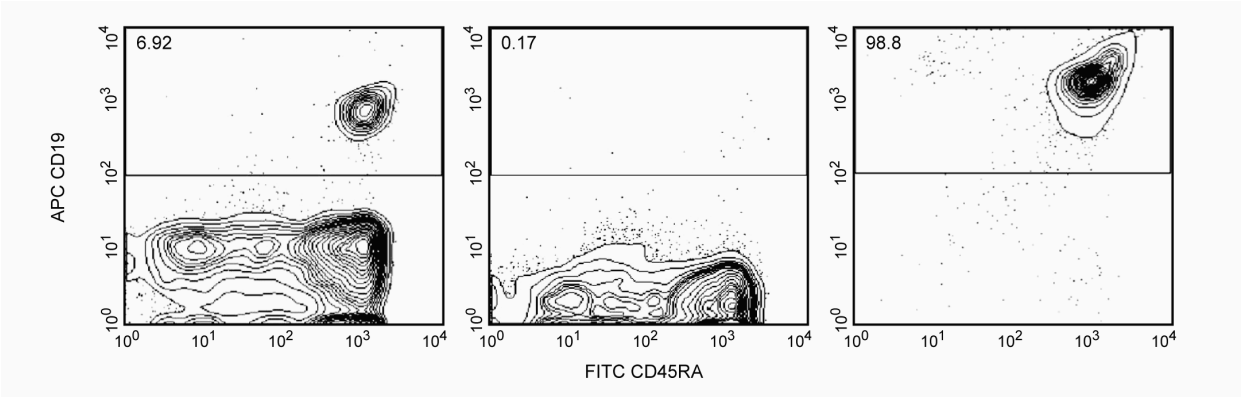
Depletions:
7. Vortex the BD IMag™ Anti-APC Particles - DM thoroughly, and add 50 µl of particles for every 1 x 10e7 total cells.
8. MIX THOROUGHLY. Refrigerate for 30 minutes at 6°C - 12°C.
9. Bring the labeling volume up to 2 to 8 x 10e7 cells/ml with 1X BD IMag™ buffer or culture medium.*
10. Transfer the labeled cells to a 12 x 75 mm round-bottom test tube (eg, BD Falcon™, Cat. No. 352058), maximum volume added not to exceed 1.0 ml. Place this positive-fraction tube on the BD IMagnet™ (horizontal position) for 6 to 8 minutes.
- For greater volume, transfer the cells to a 17 x 100 mm round-bottom test tube (eg, BD Falcon™, Cat. No. 352057), maximum volume added not to exceed 3.0 ml. Place this positive-fraction tube on the BD IMagnet™ (vertical position) for 8 minutes.
11. With the tube on the BD IMagnet™ and using a glass Pasteur pipette, carefully aspirate the supernatant (depleted fraction) and place in a new tube.
12. Remove the positive-fraction tube from the BD IMagnet™, and add 1X BD IMag™ buffer (or medium) to the same volume as in Step 9. Resuspend the positive fraction well by pipetting up and down 10 to 15 times and place back on the BD IMagnet™ for 6 to 8 minues.
- 17 x 100 mm tube: Place on the BD IMagnet™ for 8 minutes.
13. Using a new Pasteur pipette, carefully aspirate the supernatant and combine with the depleted fraction from Step 11 above.
14. Repeat Steps 12 and 13. The Combined Depleted Fraction contains cells with no bound antibodies or magnetic particles. These cells are ready for downstream applications, or they can be further enriched by proceeding Step 16.
15. The positive-fraction cells remaining in the original tube can be resuspended in an appropriate buffer or culture medium for downstream applications, including flow cytometry.
16. To increase the purity of the Combined Depleted Fraction, place the tube on the BD IMagnet™ for another 6 to 8 minutes.
- 17 x 100 mm tube: Place on the BD IMagnet™ for 8 minutes.
17. Carefully aspirate the supernatant and place in a new tube. This is the Final Depleted Fraction. The cells are ready to be processed for downstream applications.

Positive Selections:
18. Vortex the BD IMag™ Anti-APC Particles - DM thoroughly, and add 10 to 50 µl of particles for every 1 x 10e7 total cells. The amount of particles to add will vary depending on how many cells one is targeting and the cell-surface density of the antigen. Please refer to the table on Page 1 for some common examples.
19. MIX THOROUGHLY. Refrigerate mouse or rat leukocytes for 30 minutes at 6°C - 12°C. Incubate human PBMC at room temperature for 30 minutes.
20. Bring the labeling volume up to 2 to 8 x 10e7 cells/ml with 1X BD IMag™ buffer.
21. Immediately place the tube onto the BD IMagnet™ and incubate for 6 to 8 minutes.
22. With the tube on the BD IMagnet™, carefully aspirate the supernatant. This supernatant is considered the Negative Fraction.
23. Remove the tube from the BD IMagnet™, and add 1X BD IMag™ buffer to the same volume as in Step 20. Gently resuspend the cells by pipetting up and down, and return the tube to the BD IMagnet™ for another 2 to 4 minutes.
24. With the tube on the BD IMagnet™, carefully remove the supernatant.
25. Repeat Steps 23 and 24.
26. After the final wash step, remove the tube from the BD IMagnet™. Resuspend the Positive Fraction in an appropriate buffer or culture medium, and proceed with desired downstream application(s), including flow cytometry.

NOTES:
* For depletion of mouse leukocytes, tissue culture medium usually results in a slight increase in viability and recovery, when compared to IMag buffer, without reducing cell purity. We recommend that researchers run a trial comparison of media to buffer to make sure that there are no adverse effects.
** Avoid non-specific labeling by working quickly and adhering to recommended incubation times.



Depletion of T-, B-, NK-, and myeloid-lineage cells from mouse bone marrow. *BALB/c bone-marrow cells were labeled with APC-conjugated anti-mouse CD3e mAb 145-2C11 (Cat. No. 553066), anti-mouse CD11b mAb M1/70 (Cat. No. 553312), anti-mouse CD45R/B220 mAb RA3-6B2 (Cat. No. 553092), and anti-mouse Ly-6G and Ly-6C mAb RB6-8C5 (Cat. No. 553129) followed by BD IMag™ Anti-APC Particles - DM according to the accompanying protocol for depletions. The labeled cells were separated using the BD IMagnet™. Please refer to the Depletion Flow Chart to identify the separated cell populations represented in this figure. Unseparated bone-marrow cells (left panel), the Final Depleted Fraction (middle panel), and the Positive Fraction (right panel) were analyzed by flow*



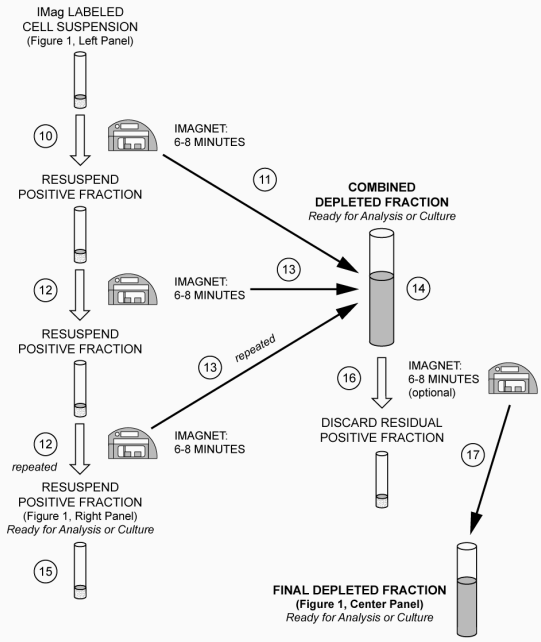
Positive selection of human B lymphocytes. *Peripheral blood mononuclear cells (PBMC) were stained with APC-conjugated anti-human CD19 mAb HIB19 (Cat. No. 555415) and FITC-conjugated anti-human CD45RA mAb HI100 (Cat. No. 555488), and then labeled with BD IMag™ Anti-APC Particles - DM. After labeling, the cells were separated using the BD IMagnet™, and the negative (CD19-) and positive (CD19+) fractions were collected as described in the Protocol for Positive Selections. Please refer to the Positive Selection Flow Chart to identify the separated cell populations represented in this figure. Unseparated PBMC (left panel), the Negative Fraction (middle panel), and the Positive Fraction (right panel) were analyzed by flow cytometry. Nonviable cells were eliminated from analysis by staining with propidium iodide, and all viable leukocytes are displayed. The percentage of CD19+ B lymphocytes in each sample is given. Flow cytometry was performed on a BD FACSCalibur™ flow cytometry system.*

Product Notices

1. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
2. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
3. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
4. BD IMag™ particles are prepared from carboxy-functionalized magnetic particles which are manufactured by Skold Technology.
5. Ficoll-Paque is a trademark of Amersham Biosciences Limited.

DEPLETION FLOW CHART

(The circled numbers correspond to the steps of the Protocol on the following page.)



POSITIVE SELECTION FLOW CHART

(The circled numbers correspond to the steps of the Protocol on the following page.)